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MICROPUNCTURE STUDIES ON THE MECHANISM OF SULFATE EXCRETION BY THE RAT KIDNEY

By

S. W. Weinstein, MAJ, MC

JULY 1969

U. S. ARMY AEROMEDICAL RESEARCH LABORATORY
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A series of free flow micropuncture experiments were performed on rats undergoing sodium sulfate diuresis. End proximal tubular fluids and ureteral urines from the punctured kidney were collected. The data indicates that tubular fluid/plasma (TF/P) ratios for sulfate remained close to 1.0 and filtrate reabsorption was quantitatively normal during sulfate infusion in the proximal tubule. Final urine analysis suggested that all sulfate leaving the proximal convolution is excreted.

The data is interpreted as evidence that sulfate is not handled by a T_M limited mechanism in the rat kidney. Rather it appears dependent upon filtration rate, proximal tubular reabsorptive rate and plasma concentration of the anion. A comparison of bicarbonate reabsorption during carbonic anhydrase inhibition to sulfate reabsorption in the rat nephron suggests greater proximal passive permeability to sulfate than bicarbonate and equally restricted distal nephron permeability.

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ABSTRACT

A series of free flow micropuncture experiments were performed on rats undergoing sodium sulfate diuresis. End proximal tubular fluids and ureteral urines from the punctured kidney were collected. The data indicates that tubular fluid/plasma (TF/P) ratios for sulfate remained close to 1.0 and filtrate reabsorption was quantitatively normal during sulfate infusion in the proximal tubule. Final urine analysis suggested that all sulfate leaving the proximal convolution is excreted.

The data is interpreted as evidence that sulfate is not handled by a T_M limited mechanism in the rat kidney. Rather it appears dependent upon filtration rate, proximal tubular reabsorptive rate and plasma concentration of the anion. A comparison of bicarbonate reabsorption during carbonic anhydrase inhibition to sulfate reabsorption in the rat nephron suggests greater proximal passive permeability to sulfate than bicarbonate and equally restricted distal nephron permeability.

APPROVED: Robert W. Bailey

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COL, MSC
Commanding

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

TABLES

MICROPUNCTURE STUDIES ON THE MECHANISM OF SULFATE EXCRETION BY THE RAT KIDNEY

INTRODUCTION

Sulfate excretion by the mammalian kidney appears governed by a classical tubular maximum (T_M) limited mechanism (1). In clearance studies utilizing dogs (2) Lotspiech demonstrated a T_M for sulfate poised at a level approximating the plasma concentration of this anion. Schwartz et al (3) were unable to show a T_M. However, technical difficulties complicated their experiments. Pitts (4) has suggested that anion competition governs the sulfate T_M.

Chloride reabsorption appears governed primarily by the relative permeability of available anions and hydrogen ion in tubular fluid. Sodium ion provides the driving force (5). Bicarbonate is apparently less permeable than chloride except in the presence of abundant hydrogen ion (5) (6).

The requirement for maintenance of electrical neutrality in tubular fluid necessitates coupled movement of cations and anions in reabsorbate. Thus for each sodium ion reabsorbed, one anion must be accounted for either via a cation exchange or anion reabsorption. Since cation exchange and anion reabsorption are probably passive processes (5), relative permeabilities of the membrane to the various particles should govern their behavior.

With these thoughts in mind, the experiments included in this report were performed. Sulfate was infused intravenously to produce a diuresis in rats. End proximal tubular fractional reabsorption of filtrate and tubular fluid concentration of chloride were measured. The data indicates isotonic reabsorption of sulfate occurs in the proximal tubule under the conditions of these experiments. Distal nephron sulfate reabsorption is in contrast close to zero. The data thus indicate lack of a T_M for sulfate in the rat.

METHODS

White Charles-River rats weighing 315-360 gms were anesthetized with Inactin 80 mg/kg. The trachea and both jugular veins were cannulated with polyethylene tubing. The left kidney was prepared for micropuncture as previously described (5) (7). The left ureter was cannulated with PE 10 polyethylene tubing. The surface tubules were transilluminated via a Zeiss Diascleral Cone adapted to a Zeiss Low Voltage Illuminator. No replacement fluid was given during the surgery.

One half hour before collections were commenced, an intravenous infusion of 0.5M $NA_2\ SO_4$ was started at 0.05 ml/min. Carboxyl C14 Inulin 10 μc/ml was contained in the infusion fluid. Just prior to collections the infusion rate was reduced to 0.025 ml/min.

End proximal tubular segments known to represent portions 50-60% of proximal length were selected (8) and free flow samples collected in sharpened single lumen pipettes utilizing Sudan Black stained light mineral oil blocks to prevent backflow contamination from distal portions of the tubule (15). A Leitz micromanipulator was utilized to perform the punctures.

Tail blood was collected before and after each puncture. Collection time and Lissamine Green transit time (8) to the puncture site were determined for each tubular fluid sample. Urines were collected from the ureteral cannula under oil in preweighed vials. Urine volume was then determined by weight. At the termination of the experiment a vena caval blood was collected.

Tubular fluids were measured for total volume in constant bore capillary tubing with an eyepiece micrometer. Alequots of the fluid were analyzed for chloride via a modification of the electrotitrimetric technique of Ramsey, et al (9) (10). The remainder of the fluid was transferred to a Beckman Dioxane Cocktail (11) for C 14 counting in a Beckman Model 200 B Liquid Scintillation Counter.

Plasma and urine were counted in a similar manner. These were also analyzed for chloride via a Cotlove Chloridometer and sodium and potassium via flame photometry.

Tubular fluid to plasma ratios for inulin (TF/P_{In}) and chloride (TF/P_{Cl}) were calculated, as were inulin clearances (C_{In}), sodium, potassium and chloride excretion rates, and individual nephron filtration rates.

Since techniques for sulfate measurement were not available, estimates of plasma and urine concentrations were calculated. Assumptions in these estimates were that plasma bicarbonate concentration was 25m M/L and other anions excluding sulfate and chloride approximated 10 meq/L. Since the sum of plasma cations equals the sum of plasma anions sulfate could be calculated from -

$$P_{Na^+} + P_{K^+} = P_{Cl^-} + P_{HCO_3^-} + P_{SO_4} + P_{other}.$$

For estimates of urinary sulfate excretion the sum of sodium and potassium excretion was utilized since the urine was almost entirely chloride free.

RESULTS

Table I presents the tubular fluid data. TF/P_{Cl} values increase in these samples to the same extent as controls previously reported by this investigator (5). Nephron filtration rates and TF/P_{In} are within normal limits when compared to non-sulfate infused rats (5). Figure 1 presents the inulin data as a function of transit time to the pipette. Included are control data obtained during 0.1 ml/min. intravenous infusion of isotonic saline. No difference exists between the slopes for control and sulfate data. The half time for reabsorption is 9-10 seconds, a value in the range of the half time of 9.2 seconds determined in this Laboratory by the split droplet microperfusion technique utilizing isotonic saline (7).

Table II presents a summary of measurements made on the ureteral urine and plasma compared to control data collected during 0.1 ml/min IV isotonic saline. C_{In} was within normal limits compared to a previous control mean of 7.7 ml/min (12). Sodium and potassium excretion rates were 14.33 μEq/min and 2.74 μEq/min, respectively, compared to control values of 14.33 μ Eq/min and 1.86 μ Eq/min, values significantly elevated above control. Urine volume was .0321 ml/min and U/P_{In} was 60.

Figure 2 presents a plot of TF/P_{Cl} as a function of TF/P_{In}. Each point represents a single sample. An increment in TF/P_{In} correlates with a concomitant increase in TF/P_{Cl}. This suggests a direct proportion between fractional filtrate reabsorption and tubular fluid chloride concentration.

Figure 3 is a graph representing end proximal TF/P_{In} vs. calculated filtered over excreted sulfate. Each point represents tubular fluid and urine samples simultaneously collected. The line represents a 1 : 1 direct correlation.

Table I

Proximal Tubular Data

	Transit Time Seconds	TF/P_{In}	TF/P_{Cl}	Nephron GFR m µl/min
	8.6	1.26	1.11	34.4
	11.0	1.93	1.11	24.0
	7.5	1.34	1.08	35.2
	9.0	-	1.08	-
	9.5	1.43	1.18	19.5
	10.2	1.46	1.16	21.6
	8.0	1.78	1.15	38.5
	8.5	1.96	1.16	29.2
	10.5	2.89	1.18	37.5
	10.0	2.36	1.19	35.2
	13.0	3.99	1.21	56.4
	15.0	4.96	1.19	46.7
	16.0	4.55	1.20	40.4
	16.0	3.65	1.17	19.5
Mean ± SEM	10.9 ± 0.8	2.58 ± 0.34	1.16 ± 0.01	33.7 ± 2.9
Control ± SEM (5) (12)	8.8 ± 0.5 (18)	1.82 ± 0.14 (23)	1.15 ± 0.02 (8)	31.3 ± 3.4 (17)

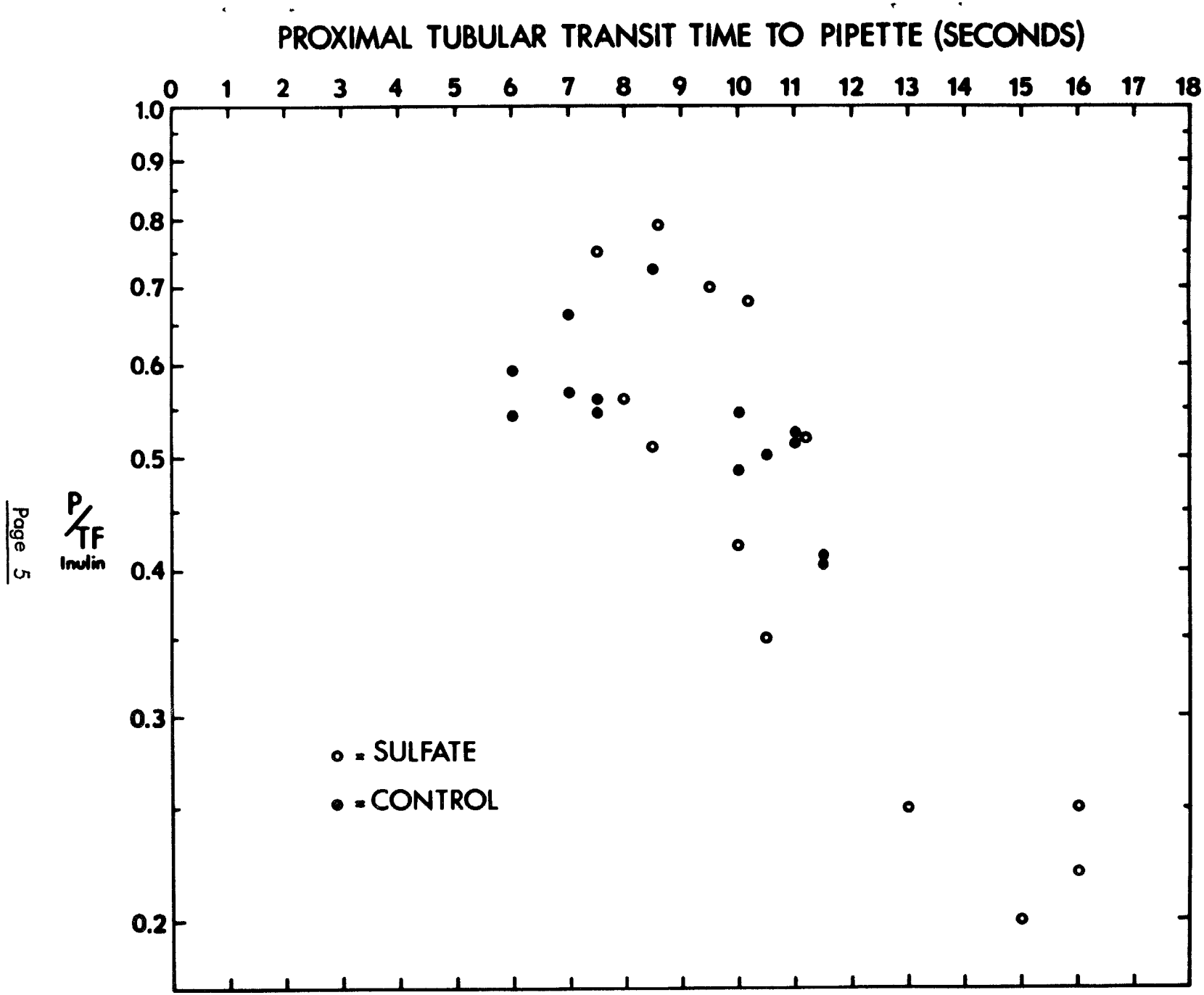
PROXIMAL TUBULAR TRANSIT TIME TO PIPETTE (SECONDS)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
P/TF Inulin
o = SULFATE
● = CONTROL

Figure 1

Table II

Final Urine Data

	C_{In} ml/min	Urine Volume ml/min	U/P_{In}	$U_{Na}V$ μEq/min	U_KV μEq/min
	10.4	.0169	97	8.72	3.28
	11.1	.0145	120	8.16	3.15
	9.2	.0130	111	7.83	2.82
	10.3	.0129	126	6.49	2.80
	10.8	.0523	35	23.71	2.92
	14.5	.0461	53	22.00	2.94
9	7.6	.0344	37	16.69	3.04
	8.2	.0334	42	15.10	2.85
	8.1	.0358	38	17.50	2.86
	8.3	.0430	35	17.89	2.65
	9.5	.0409	42	16.32	2.38
	8.1	.0381	38	14.48	2.29
	7.8	.0384	36	14.36	2.42
	5.0	.0306	29	11.38	2.01
Mean ± SEM	9.2 ± 0.57	.0321 ± .003	60 ± 9.3	14.33 ± 1.36	2.74 ± 0.09
Control Mean ± SEM (12)	7.7 ± 0.42	.0107 ± .003	82.6 ± 11.6	1.28 ± 0.35	1.86 ± 0.23

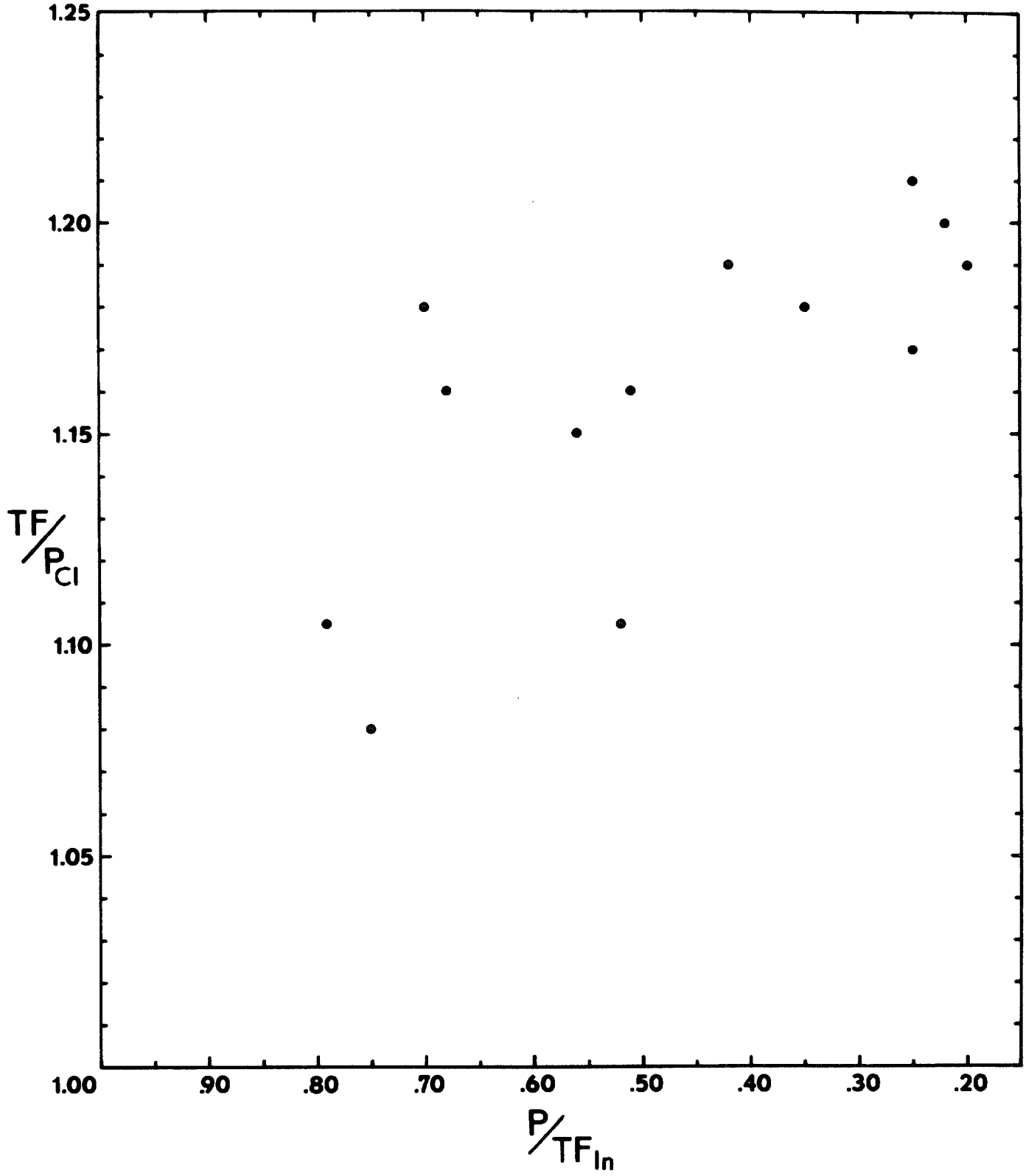

Figure 2

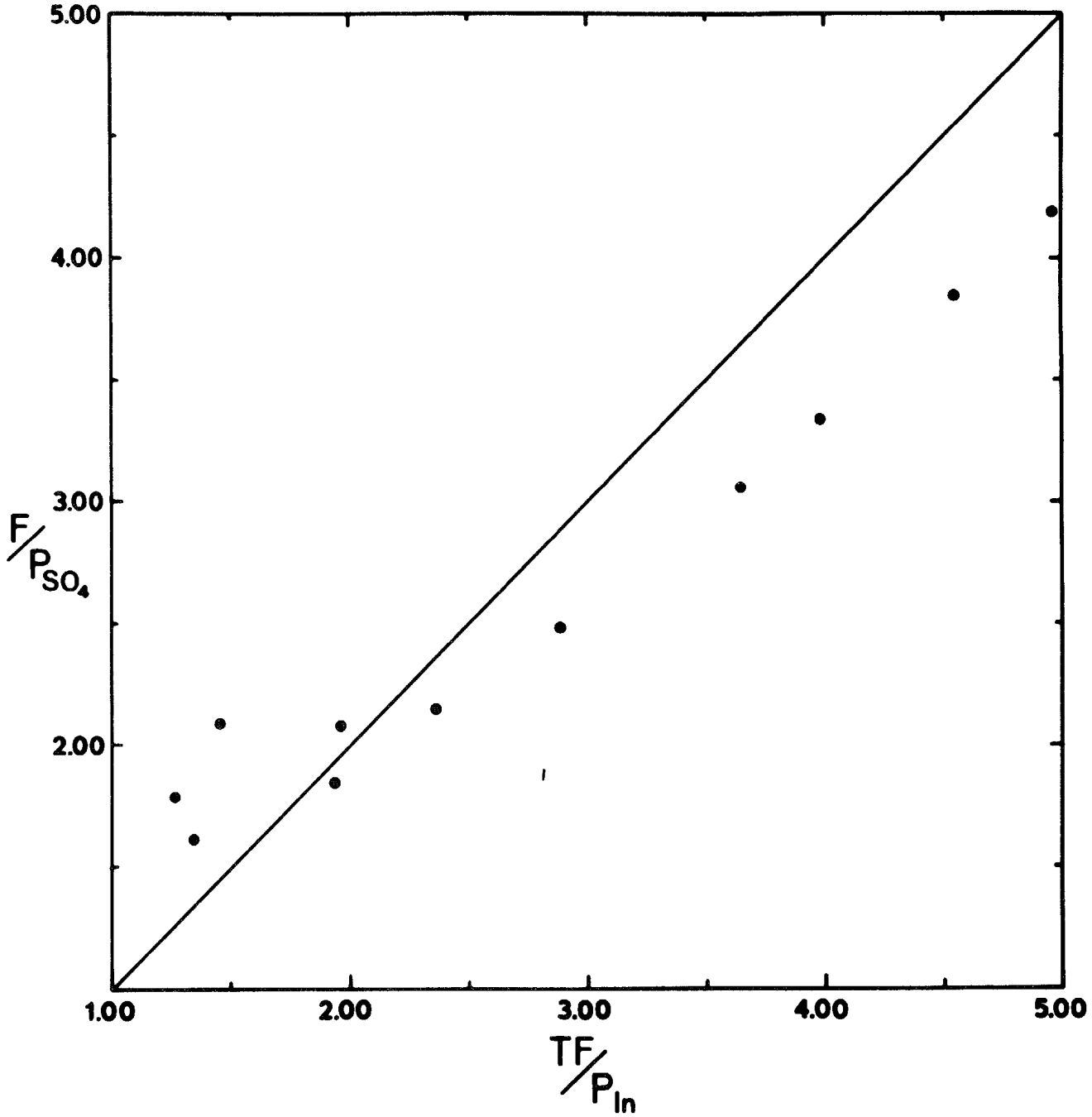

Figure 3

The data closely approximate that line.

DISCUSSION

These results provide evidence suggesting that sulfate reabsorption in the proximal tubule of the rat kidney is not T_M limited. The chloride ratios from the end proximal samples continue to rise quantitatively to the same degree as during control conditions. If proximal sulfate reabsorption were significantly limited, its concentration might increase to exceed plasma values as filtered water was reabsorbed. Since bicarbonate reabsorption must continue in the presence of normal hydrogen secretion, the rise in sulfate would replace the anion deficit produced by dissipation of bicarbonate. Chloride would either remain close to its plasma concentration or possibly fall below that level. Thus it is concluded that sulfate concentration did not rise significantly above plasma levels. The correlation between TF/P_{In} and TF/P_{Cl} lends further support to this conclusion as one would anticipate a fall in chloride or minimal rise with increased filtrate reabsorption if sulfate concentration were increasing in tubular fluid.

Another mechanism for manifesting decreased proximal tubular sulfate reabsorption would be a decrease in the fractional and/or absolute reabsorption of filtered volume. Nephron filtration rate and proximal fractional reabsorption were within normal limits during sulfate infusion. Thus volume reabsorption was not reduced. Since TF/P for sulfate approximates 1.0 and volume reabsorption is normal in the proximal tubule, sulfate must be readily reabsorbed in this segment at a concentration close to that in plasma.

The measurements in ureteral urine from the experimental kidney support and extend the micropuncture data. As indicated in Figure 3, the fraction of sulfate reabsorbed in final urine closely approximates the fraction of filtrate reabsorbed in the end proximal tubular segments. Since the sulfate TF/P approaches 1.0 in these segments the fraction of filtered sulfate and water reabsorbed proximally must be equal. Any further sulfate reabsorption along more distal portions of the nephron should be reflected in the ureteral urine. The measured fraction of filtered sulfate reabsorbed in ureteral urine and in end proximal tubular segments are nearly equal. Thus little or no net sulfate reabsorption seems to occur in the distal nephron in these experiments.

This would appear in conflict with the data of Lotspiech (2) who clearly demonstrated a T_M in dogs for sulfate at plasma sulfate concentrations well

below those noted in the present experiments. Schwartz, et al (3) were unable to demonstrate a sulfate T_M in dogs. They observed a continued reabsorption of sulfate over a wide range of plasma concentration of this anion. Since they used a single intravenous injection of sodium sulfate, constant plasma levels of the anion were not achieved. This rendered a more quantitative analysis of the data difficult.

These micropuncture results are not consistent with a T_M for sulfate in the rat. They suggest that sulfate reabsorption is determined by filtration rate, plasma sulfate concentration, and proximal tubular reabsorptive capacity. The remainder of the nephron appears almost if not entirely impermeable to sulfate since little or no net reabsorption of this anion occurs beyond the proximal convolution.

Comparing these results to measurements of bicarbonate reabsorption during carbonic anhydrase inhibition is of interest. With reduced hydrogen ion secretion bicarbonate concentration rises above plasma in the proximal tubule (13) and TF/P for chloride falls to values significantly below 1.0 (5). This coincides with fractional and absolute proximal filtrate reabsorption not statistically different from control values (5) (16). Thus proximal bicarbonate reabsorption is depressed by carbonic anhydrase inhibition. In contrast sulfate reabsorption in the proximal tubule is unrestricted. This suggests the proximal tubule is more permeable to sulfate than bicarbonate. Final urine measurements for bicarbonate excretion during carbonic anhydrase inhibition suggest that no net bicarbonate reabsorption occurs beyond the proximal tubule. Thus the distal nephron appears equally impermeable to both sulfate and bicarbonate.

In summary, the data presented provide evidence that sulfate is not handled by a T_M limited mechanism in the rat kidney. Rather, it appears reabsorbed isotonically in the proximal tubule over wide ranges of plasma sulfate concentration. Distal nephron sulfate reabsorption seems markedly limited such that most, if not all, of this anion leaving the proximal tubule is excreted in the final urine. A comparison of bicarbonate and sulfate reabsorption suggests greater passive permeability to sulfate than to bicarbonate in the proximal tubule. Distal permeability appears equally limited for both anions.

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